Alveolar macrophages and CC chemokines are increased in children with cystic fibrosis

Siobhain Brennan¹,², Peter D. Sly¹,²,³, Catherine L Gangell¹,², Nina Sturges¹,², Kaye Winfield¹,², Matt Wikstrom¹,², Samantha Gard¹,², and John W. Upham¹,²,⁴ on behalf of AREST CF²,⁵.

¹ Telethon Institute for Child Health Research, Perth, Australia.
² Centre for Child Health Research, University of Western Australia, Perth, Australia.
³ Department of Respiratory Medicine, Princess Margaret Hospital for Children, Perth, Australia.
⁴ School of Medicine, The University of Queensland, Brisbane, Australia.
⁵ Murdoch Children’s Research Institute, Melbourne, Australia.

Address for correspondence:
Professor Peter Sly
Division of Clinical Sciences,
Telethon Institute for Child Health Research,
P.O. Box 855, West Perth
W.A. 6872, Australia
Email: peters@ichr.uwa.edu.au
Phone: 61+ 8+ 9489 7814
Fax: 61+ 8+ 9489 7700

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Study question: Airway inflammation is an important component of cystic fibrosis (CF) lung disease. We sought to determine whether alveolar macrophages (AM) were involved in early CF lung disease.

Methods: Children with CF (median age 3.1 yrs) participated in a surveillance program that included annual bronchoalveolar lavage (BAL). Control samples were obtained from non-CF children (median age 3.1 yrs; n=24) investigated for persistent respiratory symptoms.

Results: Pulmonary infection was detected in 31% (16/51) & 38% (9/24) of children from the CF and non-CF groups respectively. AM in BAL were increased in CF compared with non-CF in the absence of infection (223 vs. 85 x10³ cells mL⁻¹; p=0.001) and were associated with elevations in the CC chemokines MIP3α (CCL20;355.8 vs. 46.0 pg.mL⁻¹; p<0.001), MCP-1 (CCL2; 263.5 vs. 25.3pg.mL⁻¹; ;p<0.001), MIP-1α (CCL-3; 38.2 vs. 4.9 pg mL⁻¹; p<0.001), & MIP-1β (CCL4;326.6 vs. 27.5 pg.mL⁻¹; p<0.001) Total cell counts and neutrophil numbers increased in the presence of infection however, there was no additional effect of CF.

Conclusions: Alveolar macrophages and CC chemokines are elevated in the lungs in young children with CF even in the absence of pulmonary infection. Longitudinal studies will be required to determine the clinical relevance of these findings.

Keywords: CC Chemokines, children, cystic fibrosis, macrophages.
**Introduction**

Controversy still exists over whether airway inflammation in young children with cystic fibrosis (CF) is a primary event or whether it arises only in response to pulmonary infection. Airway inflammation has been identified in young children with CF even in the absence of clinical or microbiological evidence of current infection (1-4) and has been associated with impaired lung function. (3) Understanding the mechanisms involved may result in more appropriately targeted therapy.

Previous attempts to understand the pathogenesis of airway inflammation in CF have focussed on the role of neutrophils, and the major neutrophil chemoattractant interleukin (IL)-8. Less attention has been paid to other leukocytes such as macrophages and antigen presenting cells that are likely to initiate and regulate immune responses to pulmonary pathogens. Previous studies have investigated macrophage and dendritic cells (DC) in adults with more severe disease and chronic infections (5-9). Hubeau and colleagues (10) reported an increased presence of alveolar macrophages (AM) in the lungs of late gestational fetuses with CF compared to non-CF fetuses (10). In addition, Bruscia et al (11) reported that macrophages from cystic fibrosis transmembrane conductance regulator (CFTR) knockout mice show exaggerated inflammatory cytokine responses to bacterial lipopolysaccharide (LPS); in some cases these increases were dose dependent with intermediate changes present in heterozygotes (11). These data suggest that dysregulation of macrophage recruitment and/or function may be intrinsic to CF prior to the development of pulmonary infection and may be driven in some manner by CFTR dysfunction.

Monocytes, macrophages, and DCs are recruited to the lung by CC chemokines, especially MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4) and MIP-3α (CCL20). These chemokines have received limited attention in CF, with only isolated studies on the roles of MIP-3α (CCL20)(12), RANTES
(CCL5)(13, 14) and GRO-α (CXCL1)(15). Animal studies have also demonstrated changes associated with CFTR dysfunction for MCP1(CCL2), MIP-1α (CCL3) (11).

The aims of the current study were to document the levels of CC chemokines (MCP-1, MIP-1α, MIP-1β, MIP3α and RANTES) in the bronchoalveolar lavage (BAL) fluid of young children (<6 years) with CF and to relate this to the numbers of macrophages in the lung. We hypothesised that children with CF would have higher levels of MCP-1, MIP-1α, MIP-1β and MIP-3α in BAL, than children without CF but with persistent respiratory symptoms, and that these would be associated with increased numbers of AM in lung.
Methods (see online data supplement for expanded methodology)

Subject recruitment

Fifty one preschool-aged, non-expectorating children with CF involved in the annual microbial surveillance program conducted at Princess Margaret Hospital for Children, Perth, Australia were assessed when clinically stable, defined as having no current clinical evidence of viral or bacterial infection or exacerbation of lung disease. Twenty four similar aged children without CF who were undergoing investigation for persistent respiratory symptoms, predominantly recurrent or persistent moist cough, were also included (non-CF). The diagnosis of CF was confirmed by sweat test (16) following detection by either newborn screening or by clinical presentation (3). The project was approved by the institutional ethics committee and parents gave consent for samples to be used.

Bronchoalveolar lavage processing

Bronchoscopy and BAL was conducted under general anesthesia as per the recommendations of the ERS Task Force on BAL in children (17). BAL fluid was processed for detection of bacteria, fungi and viruses and inflammation using standard methodology as described elsewhere (3, 18, 19). BAL was well tolerated in all subjects.

IL-8 was measured using a commercial ELISA (Biosource, USA; detection limit 20pg.ml⁻¹). Neutrophil elastase (NE) activity was measured by a modified enzymatic assay (20) using n-succinyl ala-ala-pro-val as the specific substrate; the limit of the assay was 0.2μg mL⁻¹. IL-6 was measured using a cytometric bead array (Biosciences, USA); the limit of the assay was 20pgmL⁻¹. Chemokines [MCP-1(CCL2), MIP-1α (CCL3), MIP-1β (CCL4), RANTES (CCL5)] were chosen as they represented known chemotactic factors for macrophages and monocytes and / or have been investigated previously in older subjects with CF. They were measured by flow cytometry using a cytometric bead array (BD Biosciences, CA, USA) with detection limits of 20pg.mL⁻¹. MIP-3α was
measured by ELISA using a commercially available kit (R& D systems, Minneapolis, USA; detection limit: 7.8 pg.ml⁻¹). Samples below the lower limit of detection were arbitrarily assigned a value of half the lower limit of detection, in order to minimise the difficulties associated with statistical analysis of zero values. All soluble mediators were measured in duplicate, and were diluted to suit the linear portion of the curves, with inter and intra-assay variation of less than 5%.

Statistics

Correlations between non-parametric variables were assessed using Spearman rank order correlation or the Pearson Product moment test for parametric data. Differences between groups were assessed by T-Test or by Wilcoxon rank test for non-parametric data. ANOVA on Ranks and ANOVA were used for comparisons between populations, and for comparisons of infection status within CF and non-CF groups for non-parametric and parametric data respectively; adjustments for multiple comparisons were made using Dunn’s Method. Values below the limit of detection for assays were arbitrarily assigned a value one half of the lowest level of detection for statistical purposes. Significant infection was defined as >10⁴CFU.mL⁻¹, while a density of 10²-10⁴ CFU.mL⁻¹ was recorded as ‘isolated colonies’. Statistical significance was achieved with a p value of <0.05. Except where indicated, group data are presented as median, with interquartile range in parentheses. Analyses were conducted using Sigmastat (Systat software inc. CA, USA).
Results

Study population

Fifty one children with CF (22 males, 29 females) with a median age of 3.07 yrs (IQR; 1.10, 4.04) were studied. The most common CF genotype was Phe508del, with 57% having 2 copies and 37% having one copy. Only 2 children did not have this mutation. Twenty four children who did not have CF (9 females, 15 males) with a median age of 3.10 years (1.79, 4.91 yrs) undergoing clinically-indicated bronchoscopy and BAL were included as a non-CF control group. The most common reason for BAL was investigation of chronic lower respiratory symptoms (83%) with the remaining children investigated for structural airway disease. BAL return averaged 32.6% in the CF group and 52.6% in the non-CF group (see online supplement for further details).

Pulmonary inflammation and infection.

Pulmonary infection was detectable in 31% (16/51) and in 37% (9/24) of CF and non-CF groups respectively. The most common organisms cultured in the CF group were *Pseudomonas aeruginosa* (n=6), *Staphylococcus aureus* (n=5) and *Haemophilus influenzae* (n=2), with a variety of other organisms seen. Forty four percent of the CF group were taking oral antibiotics at the time of the BAL. The most common organism cultured in the non-CF group were *H. influenzae* (n=4) and *Streptococcus pneumoniae* (n=2), with a variety of other organisms seen in individual children. Seventy four percent of the non-CF group were taking oral antibiotics at the time of the BAL. As a group children with CF had a significantly higher inflammatory burden with higher total cell counts [median 320 x10^3 cells mL^-1 (IQR: 210, 0.580) vs. 160 (0.110, 0.380) p=0.003], higher number of macrophages [median 311 x10^3 cells mL^-1 (IQR: 203, 544) vs. 143 (114, 378) p=0.019], higher number of macrophages [median: 237 x10^3 cells mL^-1 (IQR: 149, 301), vs. 85 (49, 166), p=0.001]), but not more neutrophils (p= 0.53) than the non-CF group. A significant correlation was seen between the numbers of macrophages and the number of neutrophils in the BAL in both children.
with CF (Figure E1, online data supplement) and those without CF (Figure E2, online data supplement). In both groups, children with respiratory infections had higher cell counts (Table 1). Children with CF also had higher levels of IL-8 in BAL [Median; 0.22 ng mL\(^{-1}\) (IQR: 0.1, 0.65) vs 0.11 (0.04, 0.29), \(p<0.001\), Figure E4 online data supplement]. Children with infections had significantly higher total cell counts (\(p=0.001\)) and number of neutrophils (\(p=0.001\)), however, there was no additional significant effect of disease (ie, CF versus non-CF). Infected children with CF had the highest BAL neutrophil counts (Table 1).

Higher levels of CC chemokines were found in the BAL of children with CF (Figures 1a-e, Figures E3a-e online data supplement), including MIP-3\(\alpha\) (CCL20; \(p=0.002\)), MCP-1 (CCL2; \(p=<0.001\)), MIP-1\(\alpha\) (CCL3; \(p<0.001\)), and MIP-1\(\beta\) (CCL4; \(p<0.001\)). There was no significant difference in RANTES (CCL5) levels between groups (Figure 1e). To account for the possibility that children defined as uninfected may have occult pulmonary infection, we conducted a separate analysis excluding children with any bacteria detected, i.e. “mixed oral flora” or “isolated colonies”. Twelve children with CF, who had available chemokine data, had absolutely no growth in BAL and had elevated BAL macrophages and CC chemokines when compared with non-CF who cultured only scant bacteria or mixed oral flora (Table E1 online supplement). The clinical features of both the CF and non-CF group, related to infection status are shown in Table E2, online data supplement. Restricting the analysis to children <18 months old, i.e. children with the lowest likelihood of having pulmonary infection confirmed elevated BAL macrophages and CC chemokines in the CF group (Table E3 online supplement).

There was a significant correlation between the number of macrophages in the BAL and MCP-1 (CCL2) in both CF and non-CF groups (Figure 2). There were also significant positive relationships between MIP-1\(\alpha\) (CCL3), MIP-1\(\beta\) (CCL4) in BAL, and the number of macrophages in the BAL
fluid of children with CF (Table E4, online supplement). These relationships were also seen in the non-CF group (data not shown). In contrast, neither IL-8, RANTES (CCL5) nor MIP3α (CCL20) in BAL was significantly associated with the number of macrophages in BAL in children with CF (Table E3, online supplement). IL-8 was detected in the majority of children from both groups; from 68.6% of children with CF and 68.2% of those without (p=1.0) with no difference in the levels between the groups (p=0.15). BAL levels of IL-8 were higher in the presence of pulmonary infection within the CF group (p=0.041) but not within the non-CF group (Figure E4, online data supplement).

Levels of IL-6, a cytokine known to regulate CC chemokine expression, in BAL correlated significantly with all CC chemokines; MCP-1 (CCL2; r=0.55, p<0.0001), MIP-1α (CCL3; r=0.55, p=<0.0001), MIP-1β (CCL4; r= 0.56, p=<0.0001), MIP-3α (CCL20; r=0.61, p=<0.0001) and RANTES (CCL5; r=0.38, p=0.006) (Table E3, online supplement). More children with CF had detectable levels of IL-6 in the BAL [27/51 (52.9%) vs 4/23 (17.4%), p=0.005] and group mean levels were higher (p=0.026), However, the influence of current infection on IL-6 levels failed to reach statistical significance in either group (CF, p=0.057; nonCF p=0.073) (Figure E5, online data supplement).
Discussion

The results of the present study demonstrate that large numbers of macrophages and elevated levels of chemokines of the CC family, i.e. MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4) and MIP-3α (CCL20) are present in the lungs of young children with CF who have mild or no clinically-apparent lung disease. The levels were not significantly increased further in the presence of pulmonary infection in the CF group, suggesting that the genetic defect in CF is likely to be at least partly responsible. In the presence of pulmonary infection the total cell counts and number of neutrophils increased in the lungs of children in both the CF and non-CF groups.

CF lung disease is characterized by intense inflammation, repeated bacterial infections and progressive lung destruction, and there is clearly a need to understand the mechanisms involved early in the disease if new forms of early intervention are to be developed. The inflammatory response to respiratory pathogens is thought to be exaggerated in both intensity and duration. Much research on the pathogenesis of CF lung disease has appropriately been directed towards neutrophil function, given that neutrophils are the predominant cell type identified in the lung. The role of other leukocytes in airway inflammation in CF has received less attention, though the results of the current study suggest that the role of macrophages should not be ignored in early CF lung disease. Lung macrophages play an important role in host defence and regulation of inflammation by interactions with inflammatory cells, including an important interaction with neutrophils. Macrophages contribute to the innate response by release of cytokines and mediators such as IL-8 and TNF-α and by facilitating neutrophil influx and efflux (21).

In the present study we noted that children with CF in the “infected group” had an increase in the absolute number of macrophages compared with the non-CF “infected” group, and that the number of macrophages was correlated with the CC chemokines measured. Interestingly, at the same time we
noted a relatively larger decrease in the percentages of macrophages in the BAL (which did not reach significance) associated with infection status. These differences (i.e. increases in absolute numbers, compared with decreases in percentages) could be a reflection of the overall inflammatory influx seen in CF compared with non-CF subjects, with relatively larger numbers of neutrophils migrating into the lung. This change in cell profile with infection may impact on the inflammatory milieu. With relatively fewer macrophages present during a pulmonary infection, there may be a relatively diminished ability to phagocytose the increasing population of dying neutrophils. Cell necrosis and the release of proteolytic enzymes is more likely under these circumstances and may contribute to the cycle of chronic inflammation that ensues. However, the relative change in the percent of macrophages present in CF versus non-CF children with the influence of infection in this study would need to be confirmed by larger numbers and serial changes within individuals as they go from an uninfected to an infected state. Specific studies targeting children before and during pulmonary infection would be problematic however; these changes may be observed opportunistically in children with CF in surveillance programs as they progress through their illness.

Animal models have provided much important information about chemokines and the role of CFTR to date. Bruscia et al (11) demonstrated that stimulated macrophages in CFTR knockout, heterozygotes and wild type mice vary, with exaggeration of cytokine and chemokine production (including CCL2), that was related to genotype. A similar relationship has been reported in patients with CF, with higher circulating levels CCL2 and IL-8 in those with genotypes associated with more severe phenotypes (13). Other animal studies have shown chemokines to have a central role in the development of allergic aspergillosis, a complication seen in CF (22). In those models CCL2 has mixed effects depending on the disease progression, other CC chemokine receptors CCR2, CCR4 and CCR5 also contribute to disease aspects including airway hyperresponsiveness, phagocytosis and killing. The role of CC chemokines has not been previously examined in early CF lung disease in a
well characterised clinically stable population. However, studies in older patients lend support to the findings from the present study. Previous studies have reported that MCP-1(CCL2) (23), but not RANTES(CCL5), is elevated in older patients with more advanced lung disease (13, 24). MIP-3α (CCL20) is elevated in primary CF epithelial cell cultures (12). MCP-1 (CCL2) and MIP-3α (CCL20) have both been reported to be elevated in BAL fluid in subjects with CF (12, 25) and in induced sputum and blood (CCL2) (23) compared with controls. MIP-1α (CCL3) has also been identified as being elevated in the tears of subjects with CF compared to healthy controls (26).

CC chemokines play an important role in both recruitment of macrophages to the lung and in the resolution of inflammation (25, 27-29). CC chemokines are regulated by cytokines such as IL-1β, TNF-α and IL-6, and this direct relationship with IL-6 was evident in the present study. MCP-1 (CCL2), MIP-1α and β (CCL3 and 4 respectively) and MIP-3α (CCL20), are known chemoattractants for activated macrophages, recruited to the lung upon exposure to C5a or bacterial proteins. These cells tend to have a different profile to the resident lung macrophages being smaller in size (30), producing different inflammatory cytokines and potentially having impaired phagocytosis (31) and, thus may be less able to assist in resolution of inflammation. Unfortunately, we were unable to isolate monocytes from BAL during this study to investigate this further. However, a recent study by Rao et al (23) demonstrated increased CCL2 in blood and sputum in older patients with CF compared with controls, with no difference in expression of the receptor CCR2 on monocytes. Macrophages play a vital role in resolution of inflammation, especially in the ingestion and clearance of apoptotic neutrophils (27, 28), and CC chemokines are intimately involved in the co-ordination of that role. Furthermore there is mounting evidence that in CF, macrophages may have impaired function in their ability to phagocytose and to clear apoptotic neutrophils (8, 9, 32) and this may be due to CFTR effects with possible mechanisms being defects in intracellular chloride levels and the inability to adequately acidify lysosomes (32). Taken together
these data suggest the hypothesis that, from early infancy, there is a high recruitment of phenotypically “small” monocytes to the airways by CC chemokines. These monocytes may not be competent to phagocytose, resulting an ineffective phagocytic response following stimulation leading to the accumulation of necrotic neutrophils. Unfortunately, the data from the present study do not shed any light on this hypothesis.

In the current study, the most striking and unexpected finding was that apparently uninfected children with CF had significantly elevated levels of the CC chemokines MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4) and MIP-3α (CCL20) in BAL fluid, compared with children with non-CF lung disease. These findings were seen both in the CF group as a whole, and also in the youngest children aged less than 18 months of age. It is unlikely that these findings can be attributed to technical factors, as a standardised method was employed for collecting BAL from all children (17) and the samples were handled in an identical manner in the microbiology laboratory. Misclassification of infection status is one possible explanation for the apparent similarity in chemokine levels between infected and uninfected children with CF. Some of the children classified as ‘uninfected’ may have had low numbers of pathogens in their lungs that were below the threshold for detection by standard culture. However, we used a very conservative definition of infection (ie. >10⁴ cfu.ml⁻¹), and there was no statistical association between isolation of low numbers of pathogens (10²-10⁴ cfu.ml⁻¹) and chemokine levels in BAL (data not shown). We cannot exclude the possibility that chemokine production and macrophage influx in CF are driven by focal infection in a region of the lung that was not sampled by BAL, and this issue would only be addressed by sampling a greater number of lobes in future studies. However, there are practical and ethical limitations to such an approach.
Some of the children with CF may have had a recent, unrecognised infection from which they had recovered, and this might explain the chemokine and macrophage data. However all the BAL samples were obtained from CF children when they were clinically stable and none had viral infections detected in BAL. In addition, there were no associations between respiratory symptom scores at the time of the procedure and BAL macrophage counts or chemokine levels (data not shown). Likewise, neutrophil numbers were not different between uninfected CF and uninfected non-CF groups (Table 1), and were relatively low, suggesting that neutrophilic inflammation does not account for the difference in chemokine levels found. Furthermore, an assessment of chemokine levels versus bacterial density failed to show any correlation with CC chemokines measured. However, with all of these considerations, it nonetheless remains possible that elevated CC chemokine production and macrophage recruitment to the lung may have been a consequence of recent undetected or current sub-clinical infection. We do have to acknowledge that our control group may not have been the most ideal. Healthy children undergoing elective surgery for non-respiratory reason may have provided a more appropriate comparison. However, performing BAL in these children is not a trivial procedure that we did not consider to be justifiable. Our group of non-CF disease controls did provide the ability to look at both currently infected and currently uninfected children for comparison with our CF group.

A variety of non-infective factors could also contribute to inflammation in CF including: impaired neutrophil clearance (9) and oxidative stress (33). Finally, it is also possible that dysregulation of chemokine production and macrophage recruitment can occur independently in children with CF and may be an early event in the initiation of lung disease. This would be consistent with the finding that lung macrophages are increased in the lungs of fetuses with CF (10), and with CFTR mouse knock-out studies (11) suggesting that monocyte recruitment and function is an issue that is worthy of further investigation as a target for therapeutic intervention.
The levels of CC chemokines found in BAL fluid found in our CF population are considerably elevated above those of the disease controls, and given the significant correlations between several chemokines and numbers of macrophages (Figure 2) and neutrophils (Table E4) in BAL, we may speculate that these chemokines are inducing recruitment of various leukocyte populations from the blood to the lung.

There have been relatively few prospective bronchoscopic studies that examine early stage lung disease in young children with CF who are clinically stable. Despite the difficulties of sampling this population, these studies are important in order to gain insight into the early stages of lung disease. The subjects in this study represent a unique cohort of children who have annual assessment of BAL from early in infancy, at a time of clinical stability and in the context of minor inflammatory lung damage. Assessment is conducted on an annual basis for the purpose of microbiological surveillance, rather than during respiratory exacerbations, which is often the case in other studies reported in the literature. As this cohort is followed up over the coming years, it will be important to determine the relationship between macrophage accumulation in the lung, CC chemokine expression and the subsequent clinical course of these children.
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References


Table 1: Inflammatory mediators in BAL fluid of Non-CF and CF groups. Data are expressed as median (interquartile range)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-CF</th>
<th>CF</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Uninfected (n=15)</td>
<td>Infected (n=9)</td>
</tr>
<tr>
<td>TCC (x10^3/mL of BAL fluid retrieved)</td>
<td>121 (104, 291)</td>
<td>548 (169, 1037)*</td>
</tr>
<tr>
<td>Macrophages (x10^3/mL)</td>
<td>85 (50, 134)</td>
<td>112 (39, 344)</td>
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<tr>
<td>Macrophages %</td>
<td>63.3 (45.7, 77.9)</td>
<td>32.7 (32.0, 46.3)</td>
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<tr>
<td>Neutrophils (x10^3/mL)</td>
<td>32.4 (11.2, 112.0)</td>
<td>155 (41.9, 656)*</td>
</tr>
<tr>
<td>Neutrophils (% TCC)</td>
<td>29.3 (13.5, 37.0)</td>
<td>38.7 (18.7, 64.3)</td>
</tr>
<tr>
<td>IL-8 (ng/mL)</td>
<td>0.10 (0.05, 0.21)</td>
<td>0.12 (0.09, 0.39)</td>
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<tr>
<td>Detectable NE [n (%)]</td>
<td>1 (7%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>NE (µg/ml)</td>
<td>0.10 (0.10, 0.10)</td>
<td>0.10 (0.10, 0.10)</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>24.75 (16.30, 29.40)</td>
<td>49.30 (15.07, 131.40)</td>
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<tr>
<td>MIP1α (pg/ml)</td>
<td>4.3 (3.05, 6.0)</td>
<td>12.2 (4.42, 68.87)</td>
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<tr>
<td>MIP1β (pg/ml)</td>
<td>24.7 (15.85, 45.6)</td>
<td>98.7 (22.10, 730.58)</td>
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<tr>
<td>MIP3α (pg/ml)</td>
<td>27.70 (14.39, 39.89)</td>
<td>545.83 (431.49, 660.16)</td>
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<tr>
<td>RANTES (pg/ml)</td>
<td>20.3 (7.25, 33.25)</td>
<td>64.2 (27.15, 129.55)*</td>
</tr>
</tbody>
</table>

Inflammatory markers expressed as median values (with IQ range), *p<0.05 between uninfected and infected subjects in each group, § p<0.05 between CF and non-CF subjects for each infection group.
**Figure Legends**

**Figure 1(a)** MCP-1 in BAL fluid recovered from CF uninfected and infected (n=36, 15 respectively) and NCF uninfected and infected subjects (n= 12, 7 respectively). Boxes represent 25th-75th percentile with the black dividing horizontal line being the median. Whiskers represent the 5th and 95th percentiles.

![MCP-1 in BAL fluid](image)

**Figure 1(b)** MIP-1β in BAL fluid recovered from CF uninfected and infected (n=36, 15 respectively) and NCF uninfected and infected (n= 12, 7 respectively) subjects. Boxes represent 25th-75th percentile with the black dividing line being the median. Whiskers represent the 5th and 95th percentiles.
Figure 1(c) MIP-1α in BAL fluid recovered from CF uninfected and infected (n=36, 15 respectively) and NCF uninfected and infected (n= 12, 7 respectively) subjects. Boxes represent 25th-75th percentile with the black dividing line being the median. Whiskers represent the 5th and 95th percentiles.
Figure 1(d) MIP-3α in BAL fluid recovered from CF uninfected and infected (n=34, 15 respectively) and NCF uninfected and infected (n=10, 4 respectively) subjects. Boxes represent 25th-75th percentile with the black dividing line being the median. Whiskers represent the 5th and 95th percentiles.
Figure 1(e) RANTES in BAL fluid recovered from CF uninfected and infected (n=36, 14 respectively) and NCF uninfected and infected (n=12, 7 respectively) subjects. Boxes represent 25th-75th percentile with the black dividing line being the median. Whiskers represent the 5th and 95TH percentiles.
Figure 2: Relationship between the number of macrophages per ml of BAL fluid retrieved from non-CF (open circles) and CF subjects (closed circles) and MCP-1 levels in BAL fluid. NCF: Slope: 0.699; intercept: 0.923, r: 0.677; CF: Slope: 0.182; intercept: 1.884, r: 0.328.